REMARKS

Entry of the foregoing and further and favorable consideration of the subject application on the merits are respectfully requested and such action is earnestly solicited.

Entry of this Amendment is proper under 37 C.F.R. § 1.116, because the Amendment places the application in condition for allowance for the reasons discussed herein; does not raise any new issue requiring further search and/or consideration, because the amendments amplify issues previously discussed throughout prosecution; does not present any additional claims; and places the application in better form for an appeal should an appeal be necessary. The Amendment is necessary and was not earlier presented because it is made in response to arguments raised in the final rejection. Entry of the Amendment, reexamination and further and favorable consideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.116, are thus respectfully requested.

Status of the Claims

As correctly stated in the Official Action, Claims 66-99 are currently pending in the present application. Claims 66-99 stand rejected.

By the present amendment, Claim 66 has been amended to clarify that the third enriched library contains elements which bind to the first mounted tissue, but not the second mounted tissue, and that the fourth enriched library contains elements that do not bind to the first mounted tissue, but that do bind to the second mounted tissue. Support for this amendment can be found in the specification at least on page 2, line 30 to page 3, line 5 ("A further purpose of the invention is to provide a method with a broad applicability as an analytical tool in studies of cell and tissue development and of antigenic phenotypes associated with tissue pathology of various conditions. ..."); page 4, line 28 to page 5, line 27; page 6, lines 8-14 ("... In either case bound structures or unbound structures are recovered in dependence of the selection intended. A selection can thus be a combination of a tissue phenotype subtractive approach, i.e., the use of both positive and negative

selection ..."); and in the Examples (*e.g.*, page 15, l. 25-16, page 2: "A mixture of filamentous phage particles ... was applied to frozen sections of small intestine or control (uterus) tissue in order to perform a negative selection step. After overnight incubation at +4C unbound phages in the supernatant were transferred to sections of colon epithelium in order to perform a positive selection step."). Claim 66 has also been amended to recite that the antibody or antibody fragment is linked to a filamentous phage, virus, polysome, or coded bead comprising antibody- or scFv/Fab antibody fragment-encoding sequence. Support for this amendment is found on page 7, lines 9-14 ("... a first library of several binding structures is liked to genetic and/or other identifying information. Preferably, the linkage between such binding structures and genetic and/or other identifying information comprises particles of a filamentous phage or of any other virus. The linkage can also comprise polysomes or coded beads, i.e. beads identified by means of coding.") and in the Examples. Dependent claims 70, 88-90, and 93 have been amended to be consistent with the amendments to independent Claim 66. Support for these amendments can be found, at least, in that recited above for independent Claim 66. No prohibited new matter is believed to have been added.

Personal Interview

Applicants gratefully acknowledge the courtesy shown by Examiners Wang and Ponnaluri at a personal interview with Applicants' representatives Mercedes Meyer and the undersigned agent on November 12, 2003.

Applicants note for the record that although the interview was initiated by Applicants, the box indicating that a separate record be provided by Applicants is not required was checked on the Interview Summary. Thus, upon best information and belief, Applicants assert that the requirements of 37 C.F.R. §§ 1.133 and 1.2 have been met.

Applicants further gratefully acknowledge the courtesy shown by Examiners Wang and Ponnaluri in subsequent telephone conferences discussing possible claim amendments.

Information Disclosure Statement

Applicants respectfully request that the Examiner acknowledge the Information Disclosure Statement filed on July 2, 2003, and return an initialed copy of the PTO-1449 submitted therewith with the next Official Communication.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 66-95 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. The Office Action questions whether the bound elements comprise the monoclonal antibody to the target and the bound elements are cleaved from the mounted tissue. The Office Action asserts that it unclear how, in step (I), a monoclonal antibody to the target is isolated or identified from the third enriched library which contains only unbound elements to both the first mounted tissue and the second mounted tissue. This rejection, to the extent that it may apply to the claims as amended, is respectfully traversed.

As noted in Applicants' previous response, bound elements comprise the monoclonal antibody or scFv/Fab antibody fragment. However, as is clear from the specification, this antibody or antibody fragment is also linked to some sort of sequence-identifying information (*e.g.*, a phage containing the nucleic acid sequence of the antibody or antibody fragment or a polysome or coded bead identifying the sequence of the antibody or antibody fragment). Once unbound antibodies or antibody fragments are removed by elution, bound elements are then cleaved from the tissue. After cleavage, the antibody or antibody fragment portion remains attached to the target tissue. The cleaved marker can be used to determine the sequence of and/or regenerate the antibody or antibody fragment. The claims now recite a "filamentous phage, virus, polysome, or coded bead comprising antibody- or scFv/Fab antibody fragment-encoding sequence" instead of "sequence identifying information. Applicants thank the Examiners for their suggestion with this and the remaining claim amendments.

Additionally, by the present amendment, claim 66 has now been amended to clarify that the **first** library is enriched for elements which **do not bind to the first mounted tissue**; the **second** library is enriched for elements that **bind the first mounted tissue**; the **third** library is enriched for elements that **bind to the first mounted tissue**, but **do not bind the second mounted tissue**; and the **fourth** library is enriched for elements that **do not bind to the first mounted tissue**, but that **do bind to the second mounted tissue**.

Applicants respectfully submit that the language of Claim 66 and its dependent claims is clear. Withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 88-91 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description. The Office Action asserts that the specification purportedly does not disclose any initial library comprising an amino acid or a nucleic acid sequence that identifies an antibody. Therefore, there is allegedly no written description for "antibody identifying sequence information." This rejection, to the extent that it may apply to the claims, as amended, is respectfully traversed.

Applicants respectfully submit that the present specification does provide written description support for this terminology. Applicants expressly disagree with the Examiner's statement that page 20 of the specification, which discloses the use of phage cDNA to determine and reproduce the structure of the molecule, "is clearly not the support for the claimed invention (sequence identifying information)." *See* Office Action, page 8. The Examiner's attention is pointed to page 7 of the specification, which describes genetic and/or other antibody-identifying information. Further, with phage display, the nucleic acid encoding the antibody is the antibody-identifying information. Thus, the presently claimed invention reflects the fact that the antibody or antibody fragments are identified via a filamentous phage, virus, polysome, or coded bead comprising antibody- or antibody fragment-encoding sequence such that the antibody can be reproduced once it is enriched in the claimed method.

As noted above, the Examples of the present specification clearly disclose the use of phage cDNA to determine and reproduce the primary structure of the molecule. Additionally, the specification on page 7 describes polysomes or coded beads, *e.g.*, for use with chemical libraries. The primary structure of the monoclonal antibody is determined, *e.g.*, from the cDNA, but a particular sequence itself is in no way part of the method. Instead, Applicants are claiming the use of differential tissue sections to isolate antibodies that are selective for a physiological state. Accordingly, Applicants respectfully submit that the present specification provides adequate written description support for the recitation of antibody sequence-identifying information.

Nevertheless, as helpfully suggested by Examiners Wang and Ponnaluri during the personal interview and in subsequent telephone conferences, Applicants have amended independent Claim 66 to recite a "filamentous phage, virus, polysome, or coded bead comprising antibody- or scFv/Fab antibody fragment-encoding sequence" linked to the antibody or antibody fragment. Dependent Claims 88-92 are further directed to filamentous phage or viruses, polysomes, or coded beads.

Withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 102(b)

Claims 66-77, 79-81, 85-87, 92, and 99 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Cai et al. (*Proc. Natl. Acad. Sci. USA* 93:6280-6285 (1996)). The Examiner argues that Cai et al. disclose a melanoma-specific antibody (V86) cloned from a phage library. The Examiner argues that tissue sections are cut from the frozen tissue cells of melanoma tumors or normal skin and used for histochemistry, following the method steps of cultured cells. The Examiner argues that the frozen tissue was exposed to the phage library, that the unbound phage was washed and the bound phage identified. This rejection, to the extent that it applies to the claims, as amended, is respectfully traversed.

a. The disclosure of Cai et al. (1995 and 1996)

Applicants again respectfully submit that the Examiner has mischaracterized the use of tissue sections by Cai et al. (1996). Cai et al. (1996) do not use the tissue sections for panning the antibody or antibody fragment libraries. Rather, Cai et al. (1996) use only **melanoma** cell lines for panning (*see* page 6281, 1st col., last bridging paragraph). They further use both the cell lines and the tissue sections for performing immunohistochemistry (*see* page 6281, col. 1, lines 2-3 and col. 2, "ELISA Assays") to confirm the specificity of the V86 antibody identified during the panning method. The Examiner argues that Cai et al. do "screen the V86 antibody library using two different melanoma cells ... (immunohistochemical staining refers to 'mounted tissue' of the instant claims)." *See* Office Action, pages 9-10. This statement is incorrect. Cai et al. (1996) are merely confirming the specificity of the V86 antibody **previously identified with panning** using cell lines, **not** performing panning on mounted tissue as in the presently claimed invention. Moreover, Cai et al. (1996) neither disclose or suggest the alternate panning (*e.g.*, expose library to "normal" mounted tissue, followed by "diseased" mounted tissue) as recited in present Claim 66.

The Cai et al. (1996) publication cited by the Examiner describes the panning of the light chains of and the characterization of antibody V86 that was previously identified in a panning procedure using immortalized cell lines (Cai et al., *Proc. Natl. Acad. Sci. USA* 92:6537-6541 (1995)). Although Cai et al. (1996) describe a panning procedure, it is directed towards screening the light chains of the V86 antibodies. Applicants respectfully submit that the method of the presently claimed invention is fundamentally different from that described in either Cai et al. (1996) or Cai et al. (1995) as discussed below.

b. Cai et al. do not disclose alternate panning or cleavage.

Cai et al. (1995) disclose the panning of peripheral blood lymphocytes (PBLs) against a tumor cell line to define those cells which bound the tumor cell line. Only those PBL cells which bind to tumor cells were used to make the phage library. Cai et al. (1995) then pan only against live melanocytes. Cai et al. (1995) do not disclose or suggest alternate panning to enrich libraries of antibodies or fragments.

The presently claimed invention permits the alternating use of different tissues (*e.g.*, benign v. malignant) to greatly enhance the detection of rare phage. Cai et al. (1995 and 1996) do not teach the differential use of mounted tissue sections to enhance the detection of specific targets as claimed in independent claim 66. Further, with regard to dependent claims 94 and 95, Cai et al. (1995) elute using E buffer, not via enzymatic cleavage as in the presently claimed invention.

Applicants respectfully disagree with the Examiner's statement that "the features upon which the applicant relies (*i.e.*, the alternating use of normal and altered tissue) are not recited in the rejected claim(s)." *See* Office Action, page 10. Again, this is incorrect. Claim 66 clearly recites that the first mounted tissue and second mounted tissue are physiologically or pathologically different. Further dependent claim 67 recites the repetition of the steps of Claim 66. Dependent Claims 71-73 further discuss various ways in which the first and second mounted tissue may differ.

c. Cai et al. do not disclose the use of mounted tissue for panning.

Cai et al. (1995 and 1996) grow cell cultures and pan in tissue flasks, not against **mounted tissue** as in the presently claimed invention. Accordingly, Cai et al. can only disclose the detection of antibodies to external cell antigens. In contrast, the presently claimed invention possesses the ability to isolate phage to any antigen (extracellular, intracellular, intranuclear, etc.) as long as it is present in a tissue section. *See* page 2, lines 18-25 of the present specification. Applicants note that perhaps no more than 1/10 of the cell's protein is located on the membrane surface, and therefore available for binding in the method of Cai et al. In contrast, the approach of the presently claimed invention exposes the entire cell antigen portfolio. Thus, Cai et al. lacks more than one integral element of the presently claimed invention.

Applicants respectfully disagree with the Examiner's statement that "the features upon which applicant relies (*i.e.*, tissue sections) are not recited in the rejected claim(s)." *See* Office Action, page 10. This is incorrect. Independent Claim 66 refers to a first and second **mounted tissue** - this is obviously referring to a tissue section. Thus, the Examiner

need not read limitations from the specification into the claims because the limitations to which the Examiner refers are already in the presently pending claims.

d. The use of mounted tissue for panning differs from the use of cell cultures.

Applicants respectfully submit that there is a significant difference between using cell line cultures for panning versus primary tissue. For example, a tumor cell line is not a reiteration of the tumor itself in every sense, so antigens expressed on the surface of the tumor cell line will be very different to those cells in the primary tumor. The use of cell lines far more frequently detects artifacts. Depending upon where the cells are selected from to generate the immortalized cell line, the phenotype of the cells selected may not be representative of other cells from the tissue from which the cells were originally obtained. For instance, the cells selected for immortalization may have a different response to trypsin and/or have different nutritional requirements. Cell lines only represent one sort of cell within a tissue. All tissue is a heterogeneous population. Tumors, for example, may have cell populations with various karyotypes (sometimes seen in a cell line) and lineages (*e.g.*, connective, epithelial, haematological tissues). This broad spectrum of cells are not promulgated in a cell line.

The cell lines used in Cai et al. (1995) are clones of one cell and do not represent the amalgam of cells obtained in a tumor sample. For example, the Examiner's attention is directed to the classification of phage types on page 6541, lines 6-15, of Cai et al. (1995), wherein only one of the three classes was melanoma-specific, one was tumor-specific (but not melanoma-specific), and one was lineage-specific. The presently claimed invention enables one skilled in the art to detect antigens that are diagnostic of the altered tissue or may be even causative to the alteration process. Thus, the presently claimed invention provides a significant advantage over the method disclosed in Cai et al.

Applicants respectfully disagree with the Examiner's statement that "the features upon which applicant relies (*i.e.*, tumor tissue) are not recited in the rejected claim(s)." See Office Action, page 11. This is incorrect. In fact, tumor cells are specifically recited in dependent Claim 77, which is included in this rejection. Further, Applicants discussed

the advantages of the presently claimed invention because Applicants have already shown above that Cai et al. do not use tissue sections for panning. The advantages further highlight the novelty and nonobviousness of the presently claimed invention.

e. The present invention overcomes significant technical difficulties.

As Applicants have previously argued, there are significant technical difficulties that need to be overcome in order to use tissue sections as in the presently claimed invention as opposed to cell lines as disclosed by Cai et al. First, antibodies bind non-specifically to the cell surface via the Fc receptor. Blocking of this reaction using antibodies to CD32 and CD16, *e.g.*, allows specific interactions to occur. Panning after such a blocking step allows for specific interactions to be detected when using a cell line. There is no such mechanism for sections, thus, the first obstacle is to eliminate non-specific interactions. A tissue section has a myriad of cell types with the stroma being particularly integrin rich. Thus, tissue sections bind cells and phage with high affinity. Example 1 of the instant specification demonstrates data regarding non-specific binding for tissue sections. Below 10^9 phage per slide, non-specific binding increased 10-fold. Thus, non-specific binding is a technical difficulty that must be overcome with tissue sections versus cell lines.

Second, the panning method described by Cai et al. used cells fixed by a single wash in glutaraldehyde, a common method. Tissue sectioning may proceed in any number of forms, mounted, held in a preserving balm such as paraffin, rapidly frozen and sectioned. The optimal fixation method must be determined for each of these processes, *e.g.*, paraffin embedded tissue is very stable but antigen exposure is adversely affected. Cryostat sections are more representative of the cell but are not easily handled. The present inventors determined whether all or any of these sections were usable within the presently claimed method and then determined the optimal fixation procedure for tissue sections. The disclosure of Cai et al. in no way aided this process as Cai et al. is not directed to tissue, only cultured cells.

Third, the tissue section used vastly affects the purification. Example 4 of the present specification shows that when the percent of antigen-positive cells are reduced in the section, a 4-fold decrease in phage binding was observed. Whole cell panning would not be expected to cause such a dilemma. Finally, the panning itself was performed by Cai et al. in a tissue culture flask making the exposure, agitation, and elution mechanically quite easy. The procedure, when "miniaturized" to be performed on a mounted section on a glass slide required considerable adaptation (*e.g.*, placement of the slide in a 50 mL Falcon tube to facilitate effective washing (*see* page 9, line 7-8 of the present specification)).

Thus, significant technical difficulties, specific to the use of tissue sections as opposed to cell cultures, were overcome by the inventors in developing the presently claimed invention.

Applicants note that novelty and nonobviousness of the present invention is demonstrated by a simple Medline search. When Medline is searched with the terms "panning" and "cell line" over the past 10 years, 143 papers are retrieved. A similar search with "panning" and "tissue section" reveals none.

Because neither Cai et al. (1995 and 1996) reference (alone or in combination) discloses or suggests each and every element of the presently claimed invention, neither reference can anticipate or render obvious the presently claimed invention. Additionally, the presently claimed invention provides significant advantages over the methods of Cai et al. (1995 and 1996).

Finally, during the personal interview, Examiner Ponnaluri indicated that the clarifying amendments of the claims are sufficient to overcome the rejections over Cai et al. Accordingly, withdrawal of this rejection is respectfully requested.

Conclusions

From the foregoing, further and favorable consideration in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

If there are any questions concerning this amendment, or the application in general, the Examiner is respectfully requested to telephone Applicants' undersigned representative so that prosecution may be expedited.

Respectfully submitted,

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Date: November 25, 2003

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